AGENTS AFFECTING RADIO-SENSITIVITY OF MOUSE TUMORS

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An interesting and confusing aspect of the treatment of cancer by x-radiation has been the varied and seemingly erratic behavior of histologically identicaltumors subjected to equal doses of radiation. Since the beginning of radiotherapy investigators have sought reasons for this difference in behavior in the field of chemical and physical agents affecting radiosensitivity - both within and without the cell. Experimental work bearing on some facets of this problem will be presented.

Effects of Physical Agents on Radiation Sensitivity - Previous Studies

A. Temperature

Theories on the effect of temperature on radiation sensitivity are nearly as old as the science of radiation itself. As early as 1906 Dr. Hart, a French physician, stated in a lecture that radiation sensitivity of a tissue was directly proportional to the temperature of the tissue at the time of radiation. This theory became known as "Hart's law," although experimental basis for this "law" was lacking. Since that time many teams of investigators have labored attempting to substantiate "Hart's Law." At present "Hart's Law" must be considered still a theory despite a substantial body of work attempting to prove his contention

In 1921 Rohdenberg and Prime concluded that virulent rat

tumors showed an additive effect of heat and radiation, whether the heat was applied prior to or after radiation. Halberstadter and Simons followed up clinical observations with a study showing skin reactions to be increased by the application of hot packs before and after radiation. This find was substantiated by Martin and Caldwell in 1922. The work of these investigators showed an increased reaction to x-radiation (radiosensitivity) in patients following the application of a hot plaster to the skin. They postulated the increased cutaneous reaction was due to the effect of temperature.

In 1924 Mottram studied the effect of lowered temperature during radiation. Using ice water irrigation of rat tails while radiating, he found an additive effect on the skin. He also noted he could lessen this additive effect by ligation of the tails while carrying out irrigation and radiation.

In 1928 Dognon, using Ascaris eggs, found the radiosensitivity of the eggs varied directly with the temperature at which they were radiated. In 1926 Wynen used diathermy to warm human skin and found the radiosensitivity of that skin was increased 30-40%. He also called attention to the difficulty of differentiating the effect of temperature and the secondary hyperemia. In 1927 Strangeways found the destructive effect of x-radiation in the chick to be inhibited greatly when

the temperature of the embryo is lowered during radiation. He noted the decreased metabolism of the embryo under these conditions as the probable cause. In 1930 Carty published a review of the already extensive literature of factors modifying the radio-sensitivity of tissues.

In 1931 Hawkins reported that heat and radiation each increased the sensitivity of guinea pig skin to the other if applied within three hours of each other. Further studies on the effect of lowered skin temperature were inconclusive. Packard in 1930 found the radiation sensitivity of drosophila was decreased by lowering the temperature at which they were radiated. However, Crabtree and Cramer (1933) found that cold increased the susceptibility of mouse tumor cells to radium. Mottram, using bean root tips, in 1935 reported increased radiosensitivity at lowered temperatures. Henshaw and Francis, also in 1935, using wheat seeds, found no variation in radiosensitivity with temperature.

In 1936 Warren published observations on cancer in mice, rats, and humans, in which it was his impression that combined fever therapy and radiation killed more tumor cells than radiation alone. In 1937 Yunoki, a Japanese worker, reported an increase in radiosensitivity of transplanted tumors with the application of heat. In 1939 Cook reported Ascaris eggs, stored at a low temperature (five degrees centigrade) for three

weeks after radiation, showed an increased survival rate of from thirty to forty-five percent. Also in 1939 Glucksmann, using tadpoles, noted that a low temperature decreased the radiation sensitivity and postulated that this might be due to a lessened number of cells entering prophase at the lower temperature. Since 1940 Evans has shown the radiosensitivity of the skin of newborn rats to vary directly as the temperature. In 1946 Schrek reported the survival of thymic cells was not affected by the temperature during radiation, but survivals could be prolonged by incubation at a lower temperature following radiation. In 1948 Patt and Swift observed somewhat similar results using frogs as experimental animals. Using whole body radiation, they found radiotoxicity was not influenced by temperature during radiation, but that incubation at a lower temperature following radiation slowed the appearance of toxic effects. However, the eventual appearance of toxic effects was not altered. In 1949 Smith and Highman observed that mice kept at 10-20 degrees centigrade for two weeks prior to radiation survived longer than controls. In 1953 Evans concluded that within the physiological range there was little effect of temperature on radiosensitivity (in tissues). In 1954 Pollard found the radiosensitivity of viruses varied greatly and directly with the temperature at which they were radiated. His results indicated a 10% increase in radiation

effects per one degree centigrade increase im temperature.

In 1955 Bachoffer and Pahl published studies showing post radiated Ascaris eggs were more sensitive to rises in environmental temperature than non-radiated eggs. They also noted there were fewer survivors if eggs were incubated at a low temperature after radiation. They postulated that this might be due to the inability of cells at a lowered temperature to metabolize toxic substances property. O'Brien and Frank in 1956 showed that cooling a rabbit's ear during radiation protected the ear against the effects of radiation while cooling the ear post radiation increased the damage to the ear. Stapleton and Eddington also in 1956 reported observations using E. Coli, showing sub-freezing temperatures at the time of radiation protected the organisms. They postulated this might be due to interference with the production of a "toxic" substance as well as a lessened diffusion of this substance.

Again In 1956 Baldwin and Narraway reported studies in insects showing little increased radiation sensitivity by heating the insects prior to radiation, but a markedly increased sensitivity to heat following radiation. In 1956 Patt reported confirmation of his work in 1948 showing that frogs survive longer post radiation if kept cold, this being due, in his opinion, to delaying the effects of radiation rather than changing them.

Effects of Chemical Agents on Radiation Sensitivity - Previous Studies

A. Oxygen

A different approach to the problem of radiation sensitivity has been seen in recent years from workers concerned with the metabolic effects of x-radiation. The work of numerous investigators, among them Donet in 1951, Burton in 1951, Allsopp in 1951, and Rajewsky in 1952 established a supplementary theory concerning the action of x-radiation on protoplasm. The classic or target theory, long accepted, states that the action of x-radiation depends upon the destruction of certain "target" areas within the cell, presumably affecting genes in this way. In 1952 Rajewsky published a review on the "Limitations of the Target Theory in Explanation of Radiation Effects."

The more recent theory of x-radiation damage postulates that in addition to the "target" action of x-rays, there is a complex series of chemical interactions within the living cell induced by the x-radiation. The above investigators have shown that one of the actions of x-rays on pure water is the production of very numerous peroxide compounds in minute amounts, most of these peroxides being active protoplasmic poisons. It is further postulated that these identical peroxides are produced within every cell exposed to x-radiation. The work of Barron and Dickman (1949) showed that SH-group enzymes are inactivated in vitro by small doses of x-rays. This inactivation was accom-

plished by oxidation of the reduced SH group necessary for the action of the enzyme. Barron, postulating that this mechanism might be the main action of ionizing radiation, suggested the action of x-radiation on cells was due to the inactivation of essential enzymes by oxidation from products produced in the interaction of x-rays and water. He also showed that lowering the oxygen tension in water lowered the action of x-rays on the thiol group of enzymes. He went further and found that the addition of catalase protected SH enzymes, presumably by destruction of poisonous peroxides.

All these observations helped to explain observations such as those of Mottram, who in 1935 noted that the tips of bean roots were rendered less susceptible to radiation damage by anerobiasis. Evans had also reported in 1942 that retarded breathing in mice while being x-rayed lessened the skin susceptibility a great deal. In 1950 Dowdy found that anoxic anoxia protected rats from what were otherwise lethal doses of radiation. He commented that since NaCN failed to reproduce the protection against ionizing radiation, the lack of oxygen itself must be the factor and not some further metabolic product. In 1952 Stapleton et al also found, using E. Coli, that the radioprotective effect of chemicals appeared to be due to removing oxygen from the cells rather than donating hydrogen ion. Since 1953 Gray has been radiating rat

tumors in vivo under an increased oxygen tension and found the sensitivity of the tumor to radiotherapy has been markedly increased. All these investigators agree the evidence points to oxygen tension within the cell as playing an important part in the radiosensitivity of a tissue, perhaps through favoring of hindering the formation of peroxides, which in turn react with vital SH enzymes.

B. SH Group Substances

In 1951 Patt and Tyree reported the results of injecting cysteine into rats prior to whole body radiation. They found cysteine protected the rats against what would have been lethal doses of radiation. Chapman followed these results in 1950 by reporting that an injection of glutathione prior to radiation gave 63.8% protection against previously lethal doses of x-radiation in rats. Patt confirmed the protective effect of glutathione in 1950, adding that under his experimental conditions cysteine, methionine, and ascorbic acid failed to give significant protection.

In 1953 Bacq and DeChamps showed B-Mercaptoethylamine to be effective in protecting against radiation sickness as induced by x-radiation. Applying these results to a rat sarcoma, Storaashi and Rosenberg in 1953 showed a regression rate of 25.6% after pre-treatment with cysteine compared to

a regression rate of 70.3% without such treatment. In 1955 Langendorf, Koch, and Hagen noted the lack of radio-protective effect of SH groups not having the cysteine-cysteamine bodies in their make-up. Nakao and Tazima also in 1955 showed the radioprotective effects of cysteine were not operating in respect to lethality or mutating in the silkworm.

In 1952 Frederic showed the concentration of SH enzymes in the skin of an entire animal to be lowered by previous localized radiation. In the recovery phase from such local radiation he found the situation to be reversed with increased concentrations of these enzymes in the epidermis. Schacter in 1952 published results showing a similar phenomenon in the plasma where he found a lowered titer of SH enzymes after radiotherapy, radiometric substances, and surgery. He postulated this lowered level was due to an increased consumption by the regenerating tissues.

Work also had been going forward on relation of SH group enzymes to x-radiation. As far back as 1931 Rapkine had reported that the inhibition of cell division by mercuric chloride could be reversed by adding cysteine compounds. Many investigators subsequently defined the role of SH group enzymes in cell metabolism. In 1952 Beck reported that trivalent arsenicals damage rat sarcomas and mouse lymphomas, the effective dose being near the maximum tolerated dose of the

substance. He also noted the protective effect of an SH compound, in this case British Anti Lewisite. This work was confirmed by Leiter, also in 1952; Peters in 1952 noted that mono-substituted arsenicals were more toxic to cells than di-substituted compounds not because of a lessened inherent toxicity, but because the di-substituted arsenicals were taken care of by the body's inherent protective system of circulating thiols such as glutathione, whereas mono-substituted arsenicals such as Lewisite penetrated into the cells with greater ease, destroying the activity of essential enzymes. Barron, in 1952, noted that mercuric chloride produced the same effect of blocking SH group enzymes within the cell as did small doses of x-radiation. Patt, again in 1952, tried to potentiate the x-ray lethality in mice by concurrent administration of chloro-mercuri-Benzoic acid, but was unable to note any change in lethality.

C. Hormones

The use of hormones to modify not only the growth, but also the x-radiation sensitivity of tumor cells occurred to workers many years ago. As early as 1933 Eicholtz reported the effect of insulin administration prior to radiation to be an increase in the radiosensitivity of experimental tumors. In 1943 Gardner reported estrogen given prior to a lethal dose of

radiation hastened the death of the animal, while estrogen given nine days prior to radiation acted as a protective agent and prolonged life. In 1949 Patt confirmed these observations and noted Benzestrol to be similar in action to estrogen. He reported that progesterone and testosterone were inactive. In 1950 the Grahams noted that Stilbestrol and some other steroids give a cellular response to radiation, using the Papanicolau technique. Also in 1950 Ellinger reported that prior administration of testosterone propionate enhanced the lethal effect of total body irriadiation in mice. He postulated the cause of this was the similar effect on potassium concentrations within the body and the actions on lymphoid tissue.

In 1953 Graham and Graham observed that alpha tocopherol and testosterone caused a decrease in cornified vaginal cells resembling the raaction seen in a favorable response to radiation. Since they had previously been able to prognosticate on the response of a gynecological tumor receiving radiation from this cellular response, they advocated giving this agent to patients receiving radiation therapy. In 1953 Ellinger published an extensive review of endocrine effects on radiosensitivity.

Orientation

The orientation of this research was to attempt to

find a method of understanding and eventually influencing clinical radiosensitivity. There appeared to be leads in this direction from the studies in the literature. It was also obvious that the field was a difficult one with many contradictory studies. Therefore it was felt that a situation as close to that seen clinically might lend itself as a screening test for any agents which might eventually point the way to a method applicable clinically.

Methods and Materials - Mice and Tumors

For the purposes of the investigation pink eyed, dba strain female mice were chosen. These mice possessed the desirable qualities of health, low excitability and light color. The light color was desirable as it greatly alleviated the difficulty of tail vein injections, the veins being more visible in a light colored mouse. The mice were obtained when 21 days old, transplanted with tumor tissue as soon as possible after arrival, and kept in boxes - four to ten in a box - until termination of each phase of the experiment or about 60 days. Each box had a layer of wood shavings on the floor which was changed once a week. Food consisted of whole grain oats and dog food pellets. The mice remained free of disease and the mortality in untreated mice was negligible.

The tumor strain was a spontaneously occuring mammary adenocarcinoma. In the course of transplantation there was some

shift in cell type away from a glandular pattern into a more medullary carcinoma. The biological characteristics of the tumor did not change appreciably, throughout the study. The tumor was transplanted through 27 generations of mice over a period of three years with a percentage take varying from 20 to 80% with an average of 60%. It was found a potent factor in the percentage take was the age of the host at the time of transplanting, the "take" being much lower in older animals.

For the donor tumor, an animal was selected with a large (20-30mm in diameter) non-ulcerated tumor. The animal was sacrificed, the tumor excised and placed in a shallow dish of normal saline. Small bits of the outer, growing section of the tumor were then injected subcutaneously by means of a trochar into the upper and outer portions of the animal's right thigh. Technique was semi-sterile and sections were taken of representative tumors to keep track of the cell type. Each tumor was transplanted into 10-20 animals depending upon its size and other technical considerations. In most cases the transplantation was completed within thirty minutes of the sacrifice of the animal.

The tumors became palpable within one week and the size of the tumors was then closely followed throughout the experiment. In all cases the tumors were measured grossly with calipers, the best approximation of the diameter being taken as the tumor size.

Photograph A

Photograph A - Measurement of tumor with calipers

Photograph B

Photograph B - Measurement of tumor with calipers

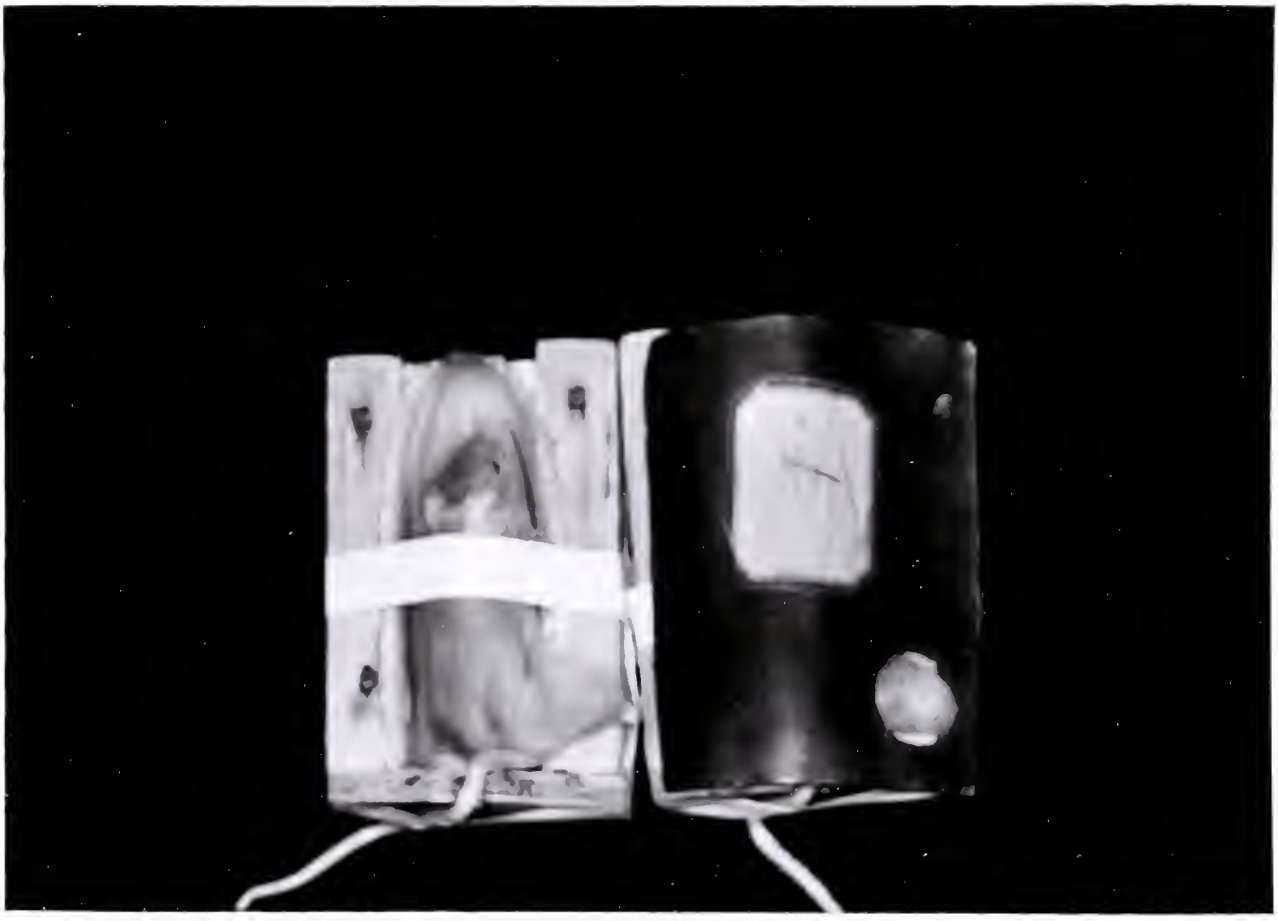
This method of following the tumors was found staisfactory, being recommended by its speed and simplicity of application. (See photographs A and B.)

It is admitted that occasionally a tumor selected for radiation might either regress spontaneously or have been an abcess. Every effort was made to avoid this insofar as possible. A control group was followed which had not been radiated. Of the thirty-three mice in this group, there was only one "tumor" which either regressed spontaneously or was never a tumor. The other tumors all exhibited marked growth in all cases exceeding 15mm in diameter before forty days. It was felt from this group (taken at random throughout the experiments) that any effect on the figures presented would be minimal.

Animals were selected for radiation when their tumors measured 4.5-6.0mm in diameter, preliminary experiments having indicated that this was the most desirable size range with these techniques. Within this size range the cure rate was relatively constant throughout.

During radiation the mice were placed in special holders. These consisted of plastic tube containers which fitted on a wooden box. Each mouse's leg was drawn out, and fixed with tape exposing the tumor. A lead sheet was then placed over the mouse, the sheet having a hole 2cm in diameter which was centered over the tumor (see photograph C).

Photograph C



Photograph C - Radiation boxes and shield

The tumors received 3000 R of radiation delivered at 520 R per minute for 5 minutes and 46 seconds. The radiation was delivered at 125 Kvp, 3.8 amp, 5.0 MA, through a 1mm aluminum filter at a distance of 1 centimeter. For most phases of the experiment it was found possible to radiate four mice at once.

After radiation the tumors were measured twice a week for a period of forty days, some mice being followed as long as sixty days post radiation. It was found that with few exceptions the tumor recurred within forty days of radiation if at all. In some cases measuring or even identification of the mouse's tumor following radiation was made difficult by radiation damage to the leg, the signs being swelling, epilation, and even ulceration. In these judgment was based on appearance, induration, and ultimately by subsequent behavior. In almost all cases tumor could be detected in one of these ways, although at times size grading was of necessity somewhat arbitrary.

The agents tested for effect on radiation sensitivity were:

A. Physical Agents

1. heat (diathermy)
2. anoxia (tourniquet)
3. anoxia plus cold (freezing with ethyl chloride)
4. incision one hour prior to radiation

5. incision three days post radiation

B. Chemical Agents

1. glutathione
2. testosterone
3. glutathione and testosterone simultaneously
4. Fowler's solution

A. Physical Agents (1) - Heat

In the study on heat it was thought desirable to heat the tumor uniformly throughout and for this reason diathermy was selected as the most promising method of applying the heat. Accordingly, small diathermy plates were constructed measuring 2 cm square. These were mounted and the mouse's leg and tumor placed between. Since the presence of any other metal mass in the vicinity adversely affected this apparatus, in order to use the x-ray machine it was necessary to add two other plates (see photograph D), at a distance to act as a shunt. These gave sufficient protection to enable the x-ray apparatus to be brought close enough for treatment without undue effect on the heating process. The summary of results of heating will be found in Chart I. It will be noticed that we were unable to pin point the temperature of the leg as closely as might be desired due to current fluctuations in the hospital line,

Chart I - Degree of Heating Obtained with Diathermy

Mouse	Temp 1	Temp 2	Temp 3
1		40.5	40.5
2		41.5	38.0
3		40.5	42.0
4		41.5	41.5
5		46.5	41.5
6		39.0	40.0
7		43.0	41.0
8		35.0	39.0
9		38.0	37.0
10		37.0	39.5
11		40.5	41.5
12		40.0	40.0
13		42.0	42.0
14	32.0	40.0	38.0
15	30.0	41.0	39.0
16	33.0	40.5	40.0
17	31.0	38.0	40.0
18	35.0	40.5	40.5
19	33.0	39.5	39.5
20	34.0	39.5	39.5
21	35.0	40.0	40.5
22	37.0	37.0	38.5
23	31.0	41.0	40.5

Chart I - Continued

Mouse	Temp 1	Temp 2	Temp 3
24	35.0	38.5	38.5
25	33.0	40.5	40.0
26	35.0	40.0	42.0
27	37.0	41.0	40.0
28	36.0	37.0	37.0
29	36.0	38.5	40.5
30	35.0	37.5	41.0
31	36.0	41.5	42.5
32	37.0	45.0	44.0
33	36.0	37.0	41.5
34	34.0	38.0	40.0
35	35.0	36.0	
36	35.0	37.0	40.0
37	30.0	39.0	41.5
38	36.0	38.5	38.0
39	35.0	41.5	41.5
40	34.0	36.0	38.5
41	36.0	38.5	39.0
42	34.0	37.0	41.0
43	35.0	38.0	40.0
44	36.0	36.5	39.0
45	36.0	38.0	39.0

Chart I - Continued

Mouse	Temp 1	Temp 2	Temp 3
46	37.0	40.0	39.5
47	36.0	40.0	38.0

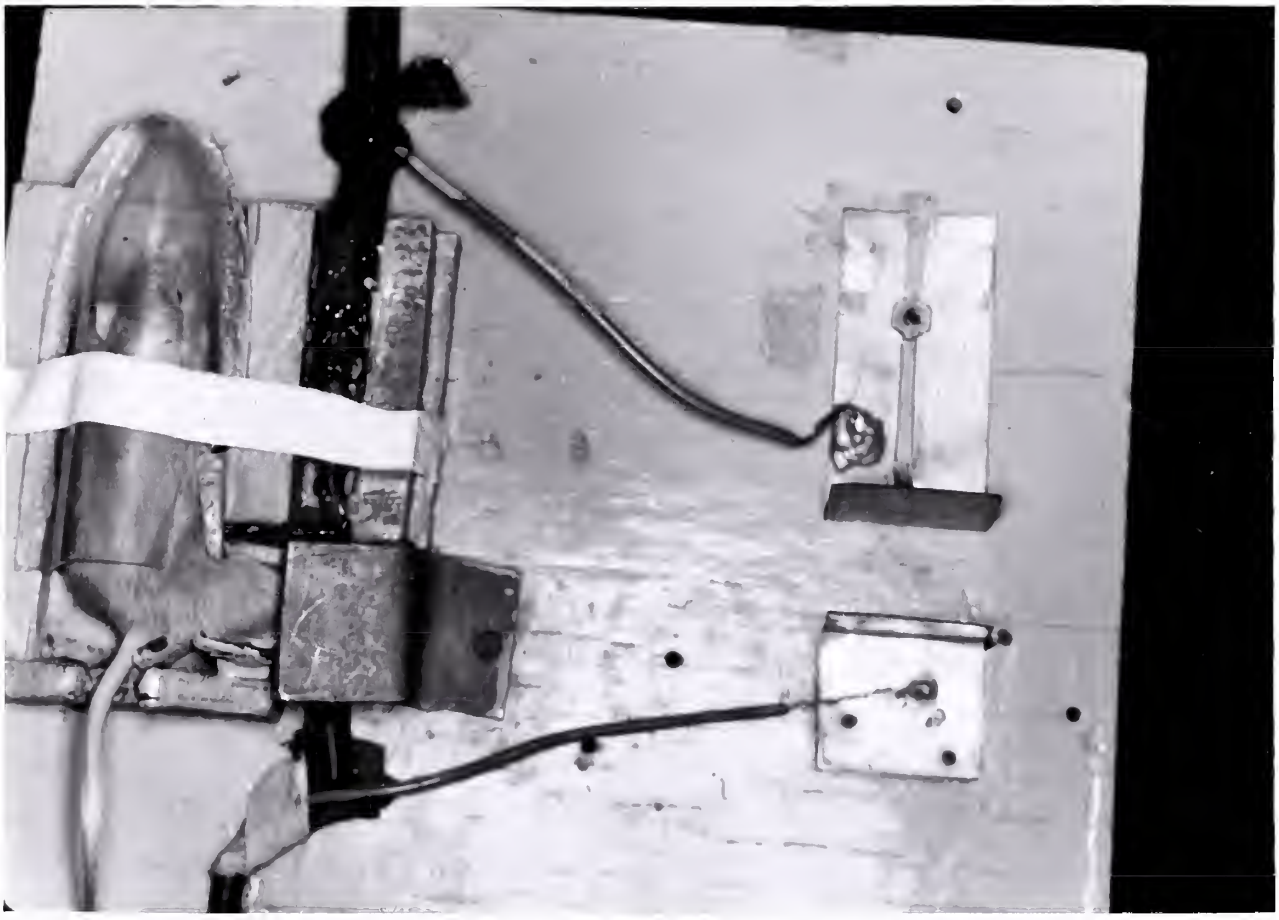
Temperature 1 is skin temperature prior to heating

Temperature 2 is skin temperature after three minutes

Temperature 3 is skin temperature after radiation

(five minutes and forty-six seconds)

Photograph D



Photograph D - Diathermy and radiation apparatus

(mouse body shield not shown)

the tiny proportion of the output of the machine we were using, and the great variations of the original skin temperature and the reactions to heating. However, it was felt that by use of a deep heating device the measurement of the skin temperature would give a close approximation of the internal temperature. Some systemic heating was unavoidable with our apparatus. However, even in the case of extreme heat applied to the leg (55 degrees centigrade) no toxic effects could be observed systemically.

A satisfactory temperature measurement device was found in an alumel-calumel thermocouple. The sensitivity of this instrument gave measurements within 0.5 degrees centigrade. To measure the skin temperature the wires were firmly pressed against the tumor, the results being duplicable within a maximum deviation of one degree centigrade. The temperature of the leg was measured in most cases before heating (Temp 1), then diathermy heating applied for three minutes, another reading made (Temp 2), and x-radiation treatment begun which lasted five minutes and forty six seconds during which time an attempt was made to keep a steady temperature. At the conclusion of the radiation a final temperature (Temp 3) reading was taken.

Physical agents (2) - Anoxia

To test the effects of anoxia on radio-sensitivity, a tourniquet (of twine) was placed around the leg of each experi-

mental animal above the site of the tumor. This was done approximately three minutes prior to radiation, remaining on through the five minute and forty-six second period of radiation, being removed immediately after radiation. The limbs were cyanotic and somewhat cool when the tourniquets were removed; however, there was no incidence of loss of leg or other untoward signs of damage.

Physical Agents (3) - Anoxia plus cold

The study of anoxia plus cold was undertaken because of lack of a feasible method to cool the tumor without causing concommitent anoxia by any method whereby the tumor would remain cool during the five minute, forty-six second time required for radiation. Preliminary experiments established that the simplest method of cooling, the application of ethyl chloride spray, was efficacious in cooling the tumor when applied without a tourniquet (see Chart II-A), but the tumor regained its original temperature rather quickly. If a tourniquet was applied prior to application of ethyl chloride (see Chart II-B), the cooling was significant for the duration of the radiation. Accordingly, it was elected to test this combination. Ethyl chloride was applied lightly to each tumor for approximately one second, then again applied three seconds later a total of ten times in order to freeze the tumor.

Chart II - A

	Original leg temp.	After Cooling	Five minutes later
1.	31	12	30
2.	33	14	33
3.	34	14	31
4.	37	12	35
5.	34	16	30
6.	35	14	37
7.	36	16	26
8.	35	18	30
9.	31	18	27
10.	37	14	24

Chart II - B (Temperatures in degrees centigrade)

	Original leg temp.	After cooling	Five minutes later
1.	34	10	20
2.	35	12	21
3.	35	12	16
4.	31	10	14
5.	37	9	17
6.	35	10	18
7.	35	10	20
8.	36	12	18
9.	34	12	19
10.	31	11	17

Physical Agents (4) - Incision one hour prior to radiation

The purpose of this study was to detect any effects on radio-sensitivity of surgery (biopsy) immediately prior to radiation. The many factors involved in wound healing (notably SH enzymes) are not well understood. This work was designed only to test the gross overall effects of surgery. An incision was made through the skin over the tumor and carried through the entire tumor. There was some bleeding, but no fatalities ensued and the incisions healed well with minimal infection.

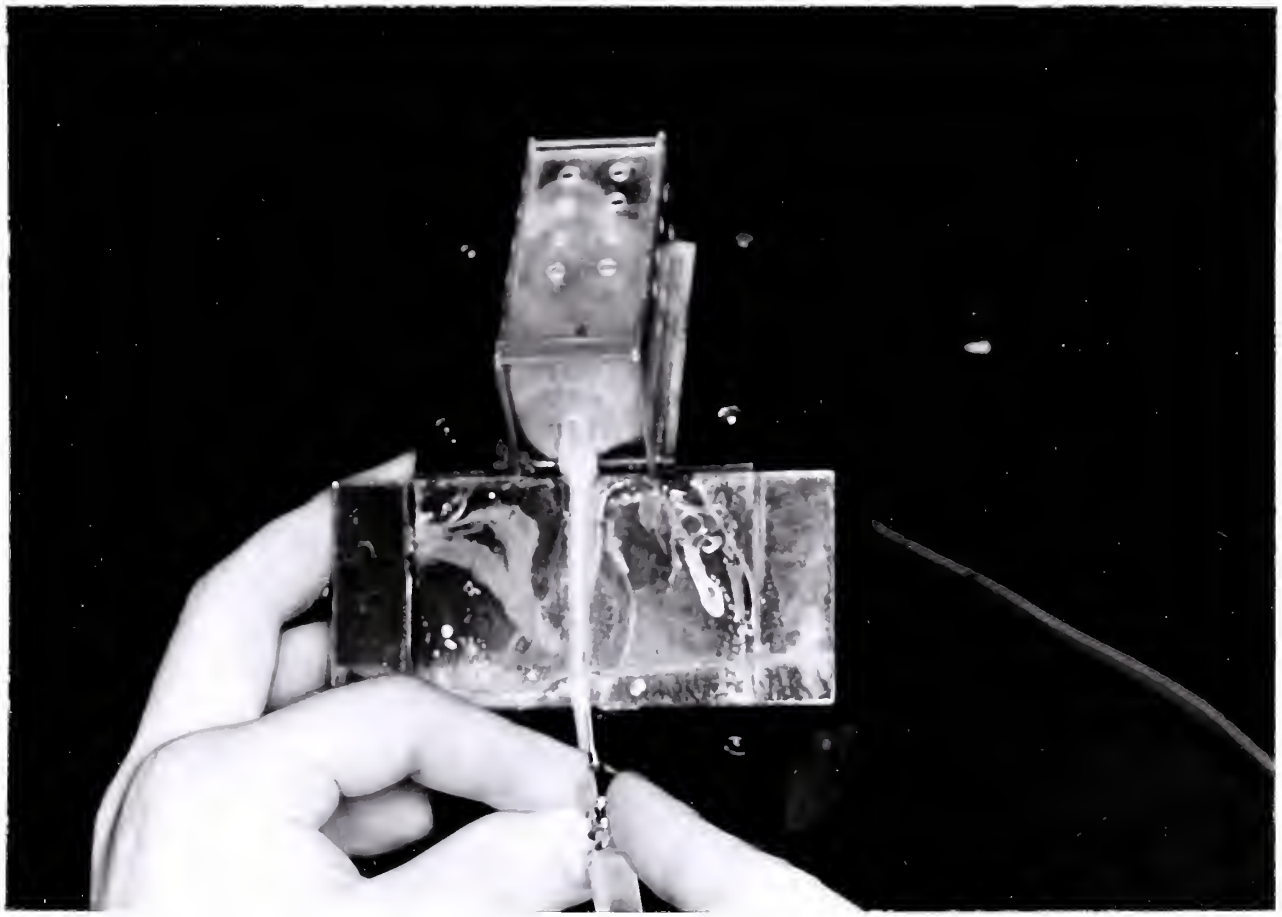
Physical Agents (5) - Incision three days post radiation

Many surgeons working with cancer have held the opinion that after radiation therapy, any type of surgery is contraindicated. It was desired to test this hypotheses insofar as local remission rate of the tumor was concerned. Accordingly, an incision was made through the skin and carried through the tumor three days post radiation. There were no fatal exsanguinations or massive infections.

B. Chemical Agents (1) - Glutathione

Glutathione was chosen to study the effect of SH substances because of its convenience and low toxicity. The solution was injected into the tail vein of each mouse within five minutes prior to radiation. For this purpose, tuberculin syringes,

Photograph E



Photograph E - Tail vein injection apparatus

number 27 needles, and a special tail vein injection box were used. (See photograph E.) Dosage used was twenty milligrams per animal of a fresh solution of three hundred milligrams glutathione to 1.05 cc 1 normal NaOH and 1.95 cc water. The volume injected was 0.2 cc. It was found that injections of this volume of saline had no effect on the radiosensitivity of controls.

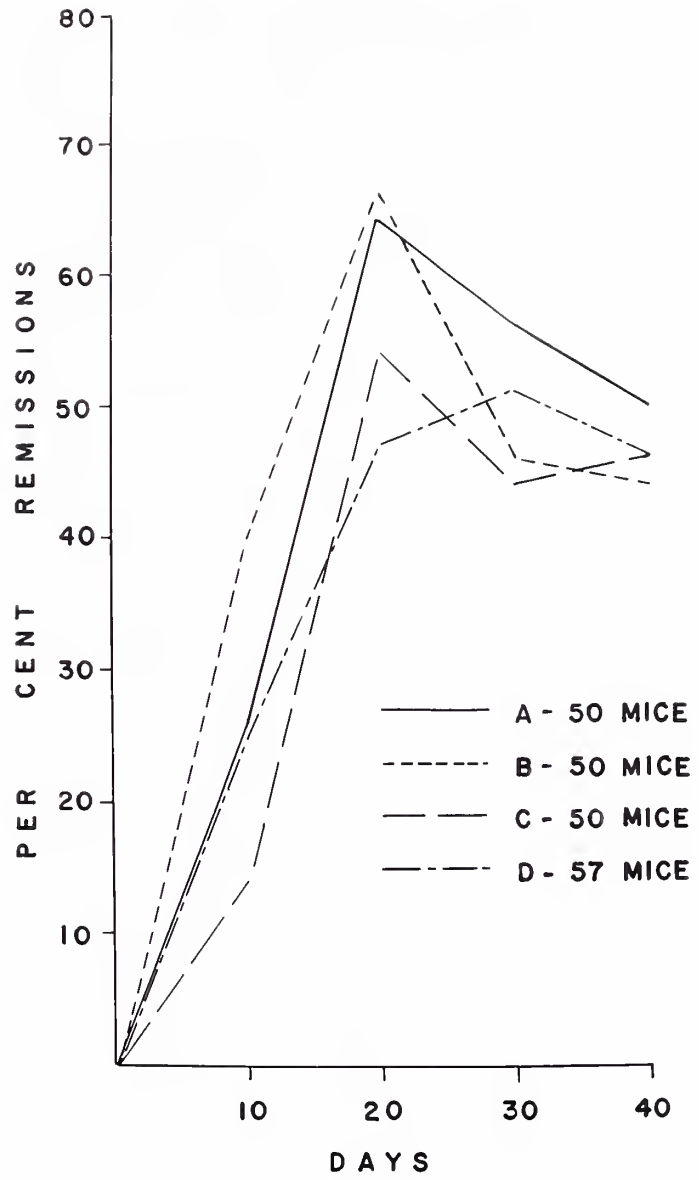
Chemical Agents (2) - Testosterone

Testosterone was chosen to be studied as a direct result of the investigations of Graham and Graham previously cited. The hormone was given 24 hours prior to radiation intra-peritoneally. Dosage was 0.05 cc of testosterone propionate.

Chemical Agents (3) - Glutathione and testosterone simultaneously

Early in the course of the work it was noted that glutathione seemed to exert a radioprotective effect on the tumors while testosterone seemed to enhance the effect of radiation at least temporarily. Therefore the two substances were given to a group of mice. Each agent was given as previously described, testosterone 0.05 cc of testosterone propionate intra-peritoneally 24 hours prior to radiation and glutathione 20 mg given intra-venously within five minutes prior to radiation.

Graph I



Graph I - Four groups of controls (radiation only) in chronological order.

Chemical Agents (4) - Fowler's solution

In the search for an SH inhibitor, the most readily available substance was Fowler's solution (potassium arsenite). The mice were given 0.2 cc of a 1/20 Fowler's solution two hours prior to radiation. (0.1 mg arsenic trioxide) This dose was experimentally determined as $\frac{1}{2}$ the LD-50 for mice of this weight. The mortality in this experimental group was markedly higher (39%) than in any other group over the prolonged course of the experiment.

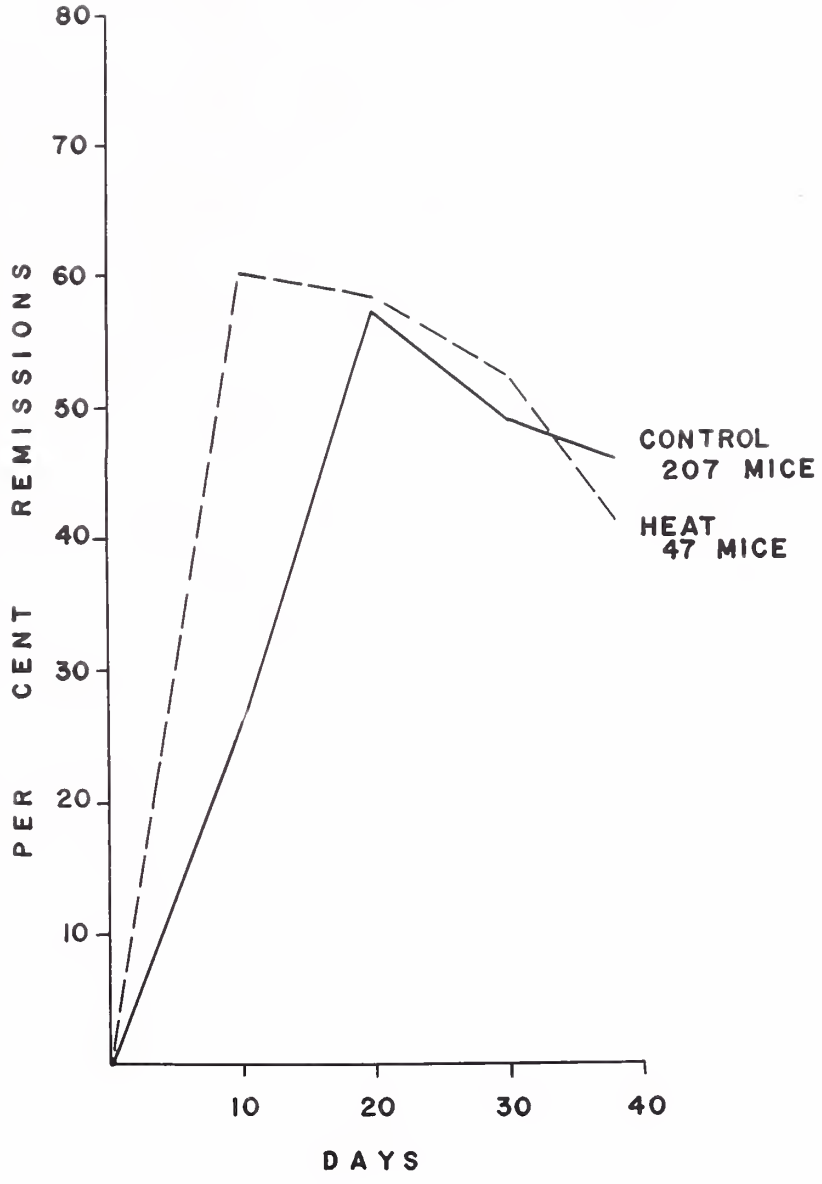
I. Results

A. Controls

The tumors in the control group were selected at random throughout the course of the experiment. Of a control group of 33 mice observed having received no radiation, there was only one remission observed. The other 32 mice had a high tumor growth rate without exception measuring over 15 mm in diameter at 40 days. The one mouse with an apparent remission was in a group of 12 of these mice which were heated without subsequent radiation.

The controls numbered 207 mice in all. Since the experiments were carried out over a three year period, a check on the behavior of the tumor in respect to radiosensitivity varying with time was felt necessary. For this purpose the tumors were divided into four groups in chronological order, each group con-

Graph II



Graph II - Effect of heat and radiation versus radiation alone

taining 50 or more mice. The results of this comparison are presented in graph I. At no point on the curves is there a statistically significant difference (greatest chi square = 3.8). The 207 mice all having received radiation alone were considered as the "control" group throughout the remainder of the presentation.

The results were tabulated as percent remissions as this figure was closer to clinical standards. A tumor which could not be seen or felt was classed as a remission. Records were kept on the size growth curves of the "controls" and experimental groups, but these were not felt to be as accurate a guide as remission rates, as size grading was of necessity arbitrary at times. The results with this method of presenting the data were equivalent and the data was presented in this manner in one graph (Graph VIII) for comparison.

B. Physical Agents

1. Heat

As was noted previously there was one observed remission in a group of 12 mice heated without radiation. This observation was attributed to experimental error, although a direct thermal effect may have been operating.

The overall results are presented in graphic form in Graph II. It will be noted that heat did not affect the over-

Chart III

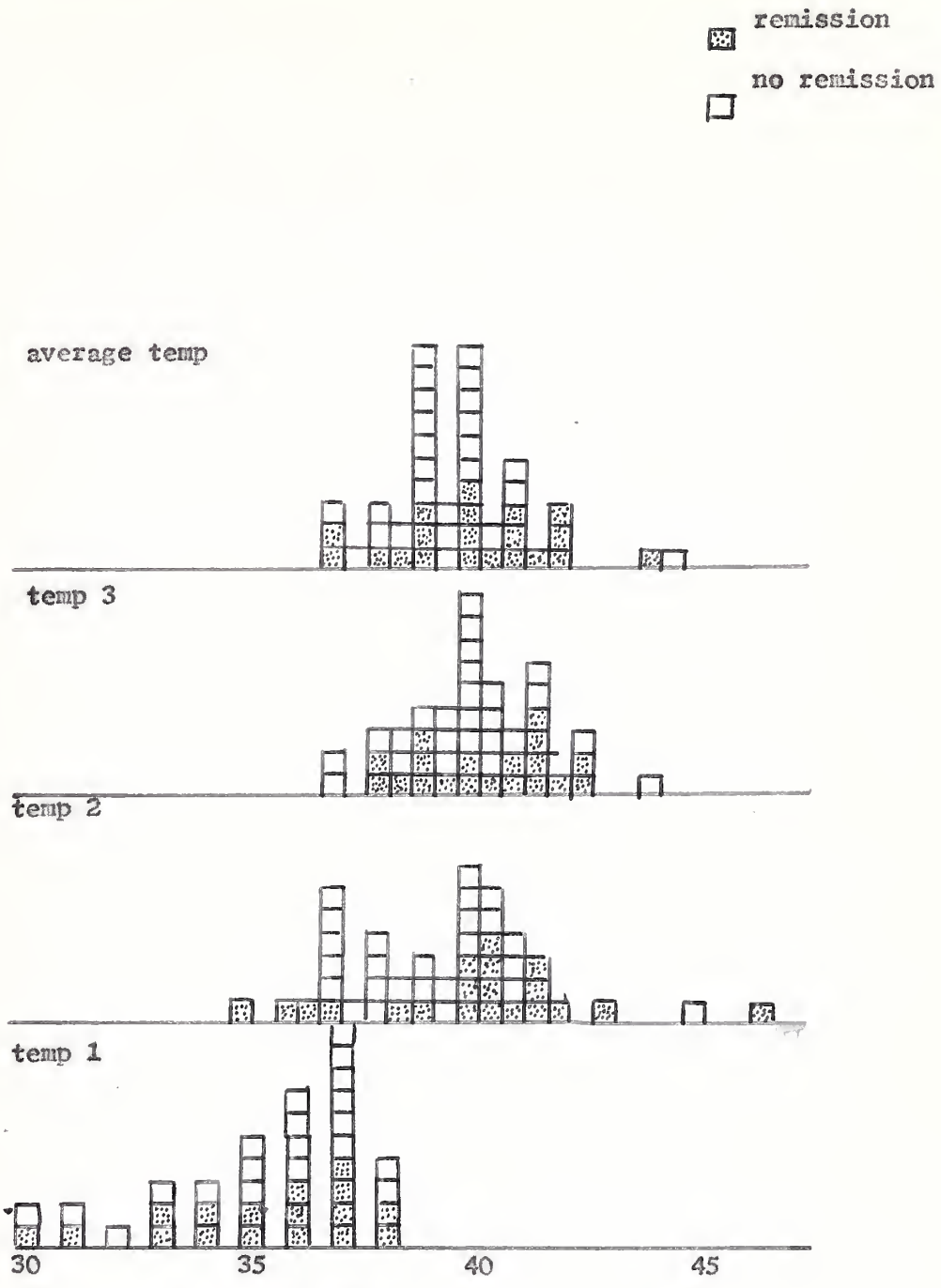


Chart III - Degree of heating (diathermy) versus remissions at 40 days

Chart IV

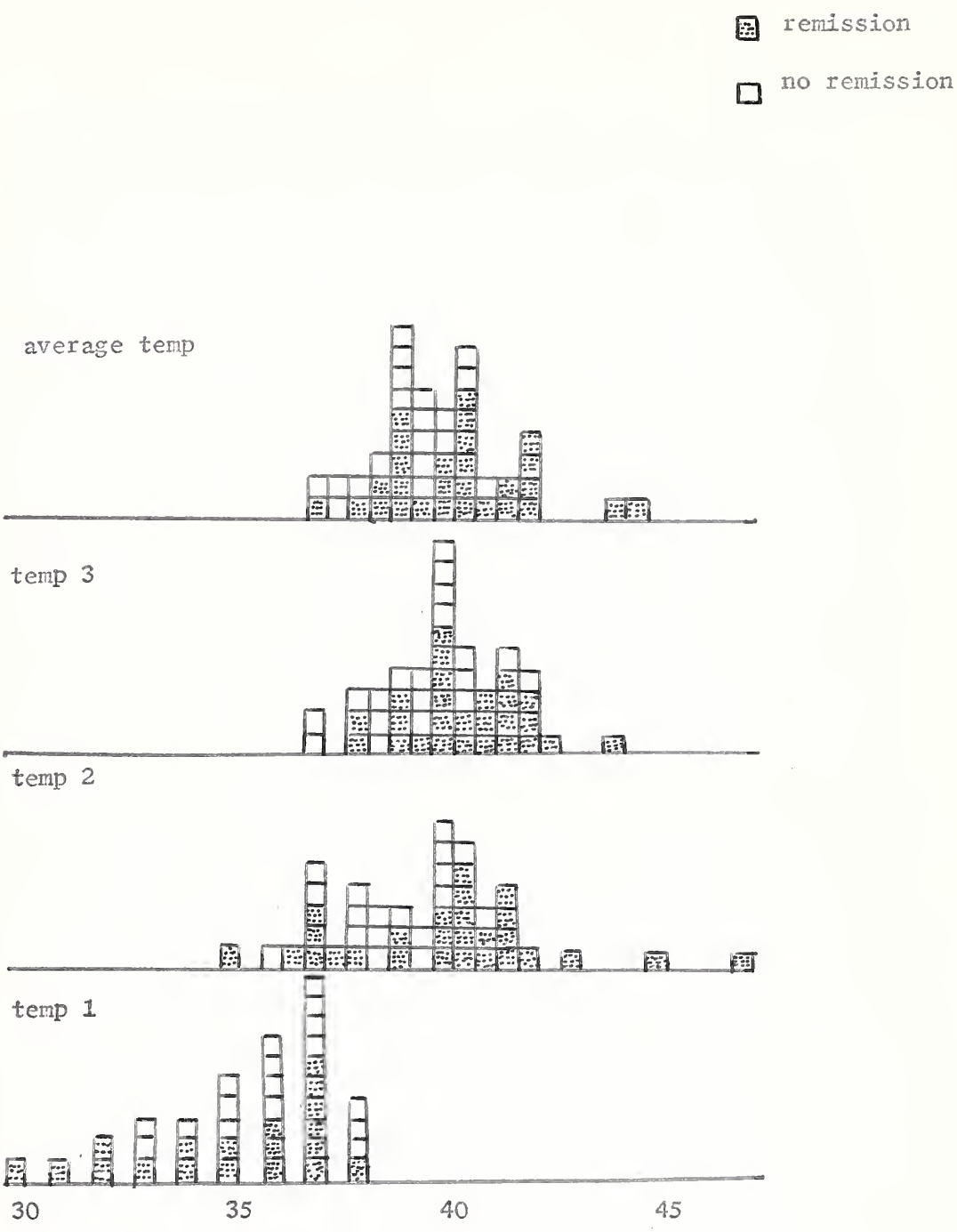


Chart IV - Degree of heating (diathermy) versus remissions at 10 days

all remission rate at the end of the experiment. However, these results do seem to indicate that there is an acceleration of the appearance of radiation damage. Damage to the tumor was the only effect consistently measured, thus the differential action of normal and cancerous tissue could only be inferred. However, approximately $\frac{1}{4}$ of this group of experimental animals suffered radiation damage to their legs so severe that the legs dropped off during the course of the experiment, most of them around twenty days. This was rarely, if ever, observed among other experimental groups. This, coupled with lack of improvement in the final remission rate, suggested this was an unfavorable form of therapy under the conditions of the experiment.

Likewise, the degree temperature to which the leg was heated seemed to have no relation to the ultimate rate of remission. The tumors are plotted as remission and non-remission versus the degree of temperature to which they were heated in Chart III. This chart shows tumors showing remission as stippled squares with growing tumors as light squares. The degrees centigrade is plotted on the abcissa. Temperature 1 is the original skin temperature of the mouse (not measured in all mice); temperature 2 is the temperature at the end of three minutes heating immediately before radiation. Temperature 3 is the temperature after 5 minutes and 46 seconds of radiation.

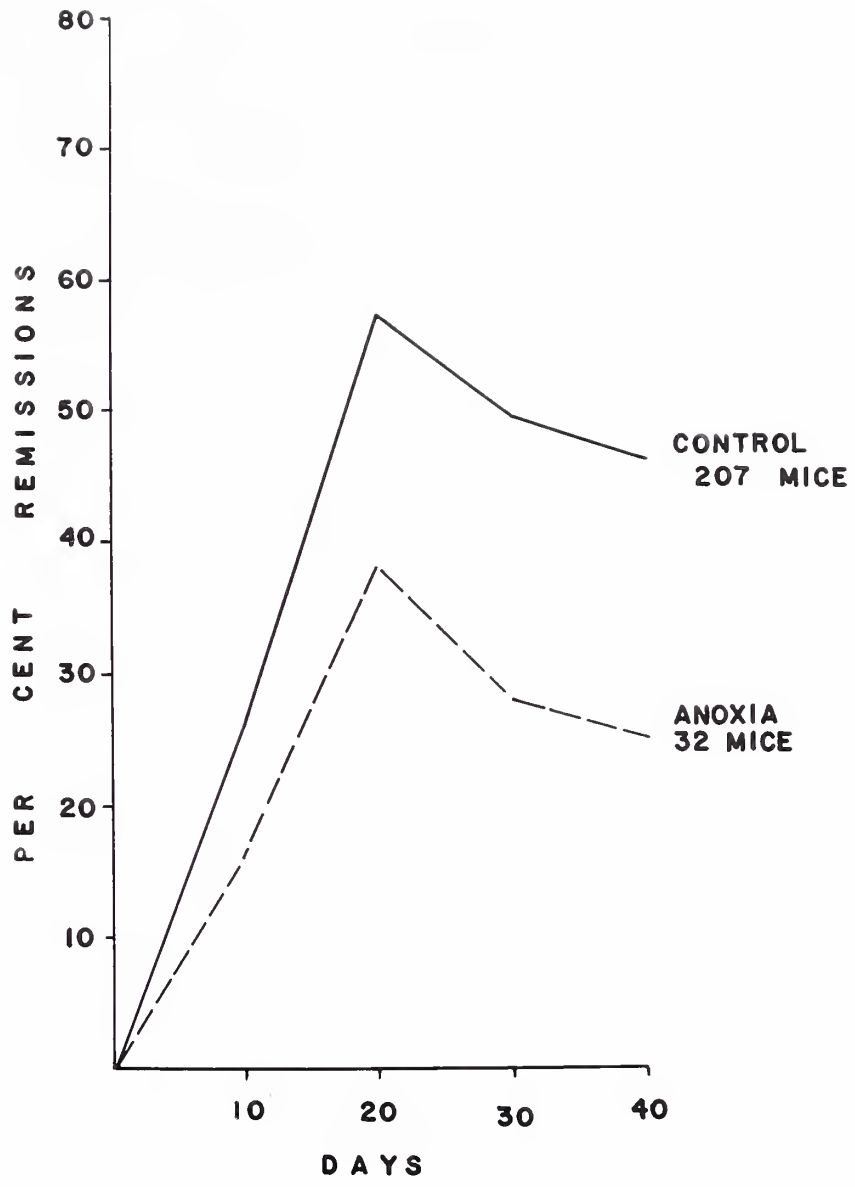
The average temperature is the average of temperature 2 and temperature 3 for each mouse, or the mean temperature during the actual radiation. Although the numbers are not significant it will be observed that there is no apparent skew of success or failure of treatment by the degree of temperature attained. This would tend to support the statement that heat plus radiation was no better than radiation alone at forty days.

As a further check of the significant difference noted at ten days between the groups (a similar chart was prepared for the ten day interval - Chart IV). This chart by contrast reveals a definite trend to remissions at this date, being proportional to the temperature to which the leg was raised. Thus the temperature would seem to accelerate damage to the tumor even if not altering the final remission rate. There is thus some evidence that heat and radiation do interact in some way.

2. Anoxia

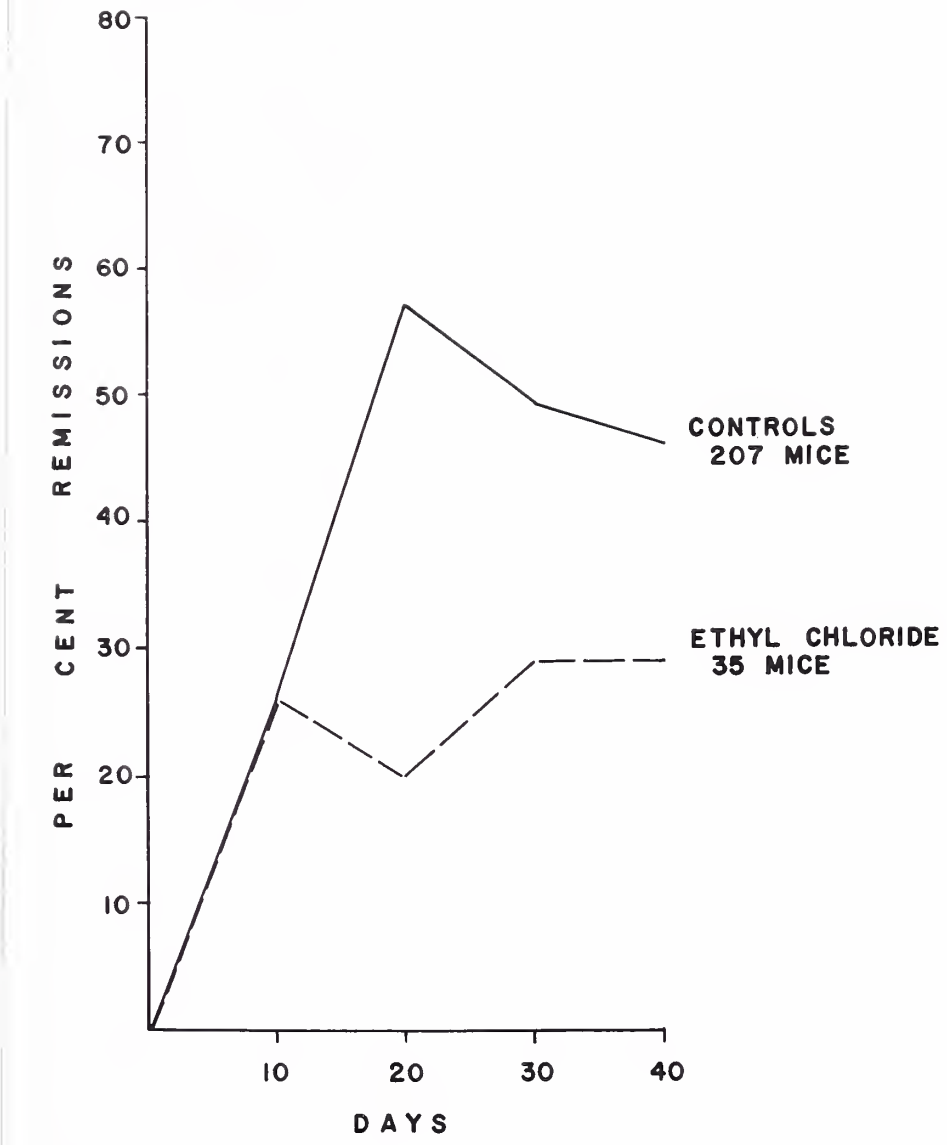
Anoxia by means of a tourniquet during radiation would appear definitely to protect tumor tissue from destruction by radiation. The results of this portion and the study are presented in Graph III. This shows a consistently lower rate of remission (which is statistically significant) after ten days - which was coupled with subjective indication of lowered normal tissue raaction (less epilation and swelling).

Graph III



Graph III - Effect of anoxia and radiation versus radiation alone

Graph IV



Graph IV - Effect of anoxia and cold versus radiation alone

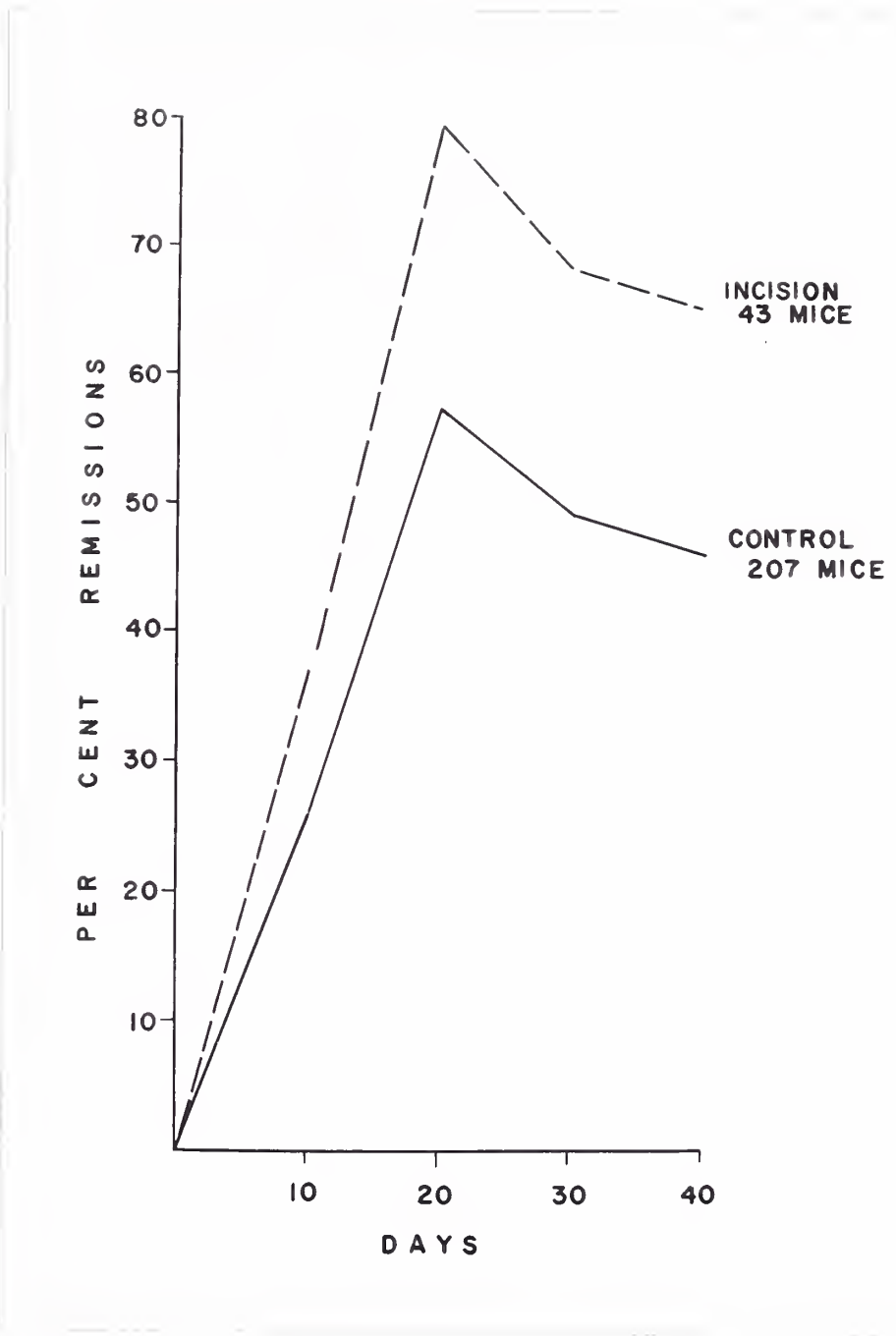
3. Anoxia plus cold (ethyl chloride)

The results are presented graphically in Graph IV. The similarity to the results using anoxia alone is apparent. The one interesting feature otherwise is a difference from anoxia alone in the rate of appearance of damage to the tumor (remission). At twenty days there was a delayed appearance of remission over that observed with anoxia alone. This difference is not statistically significant, but is suggestive. Here again is the possibility of an effect on the timing of appearance of radiation damage (remission) when the effect of cold is added to anoxia.

4. Incision prior to radiation

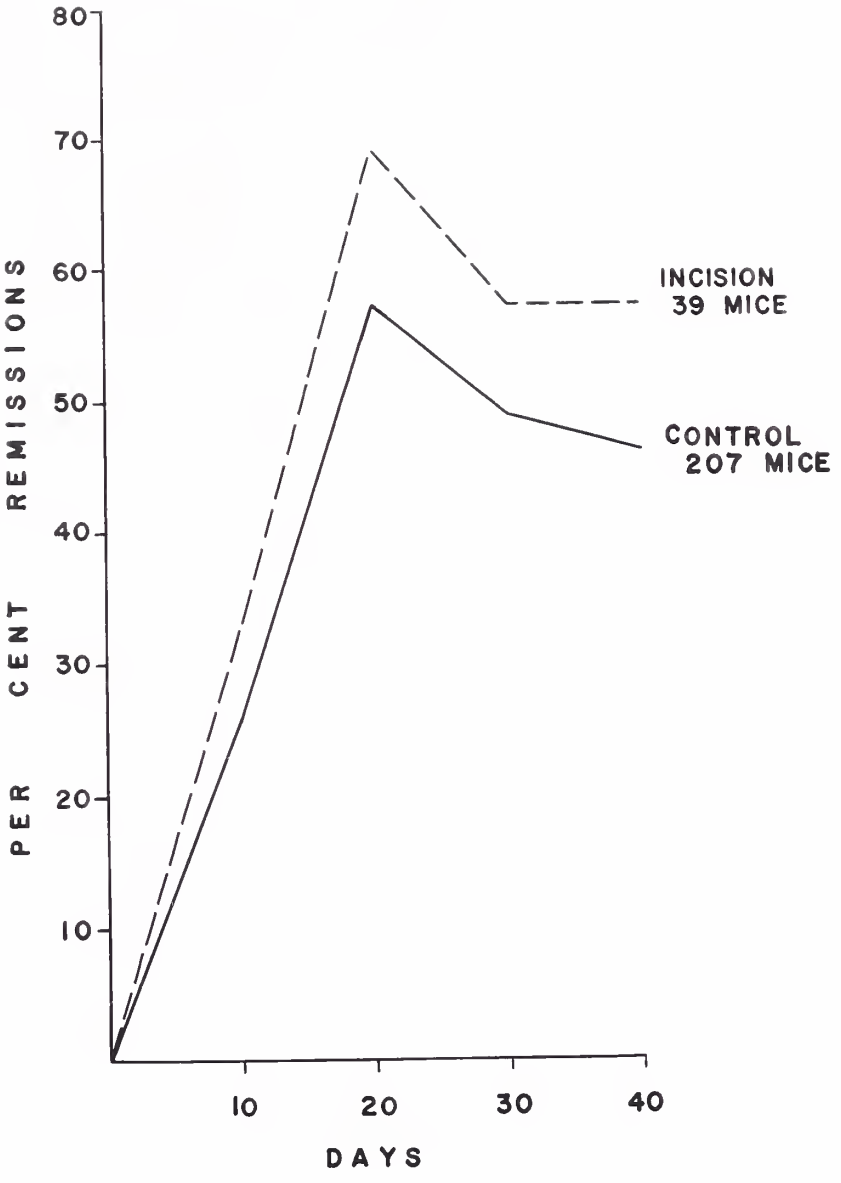
The results of mice with incisions one hour prior to radiation compared to those receiving only radiation were somewhat surprising (see Graph V). Those with incisions prior to radiation had consistenetly better remission rates, indicating an increased radiosensitivity. Mice with incision alone (15) had no cures at forty days. The results indicate a synergism between surgery and radiation under these conditions. It might be speculated that this is due to the lowered levels of SH enzymes following an attempt at wound healing. At any rate, our results indicate a probably statistically significant better cure rate with surgery prior to radiation (chi square at forty days = 5.5).

Graph V



Graph V - Effect of surgery prior to radiation versus radiation alone

Graph VI



Graph VI - Effect of surgery post radiation versus radiation alone

5. Incision post radiation

Incision post radiation (see Graph VI) produced effects which tended in the same direction as incision prior to radiation, but which were not as marked and on statistical analysis were not significant (chi square = 2.1).

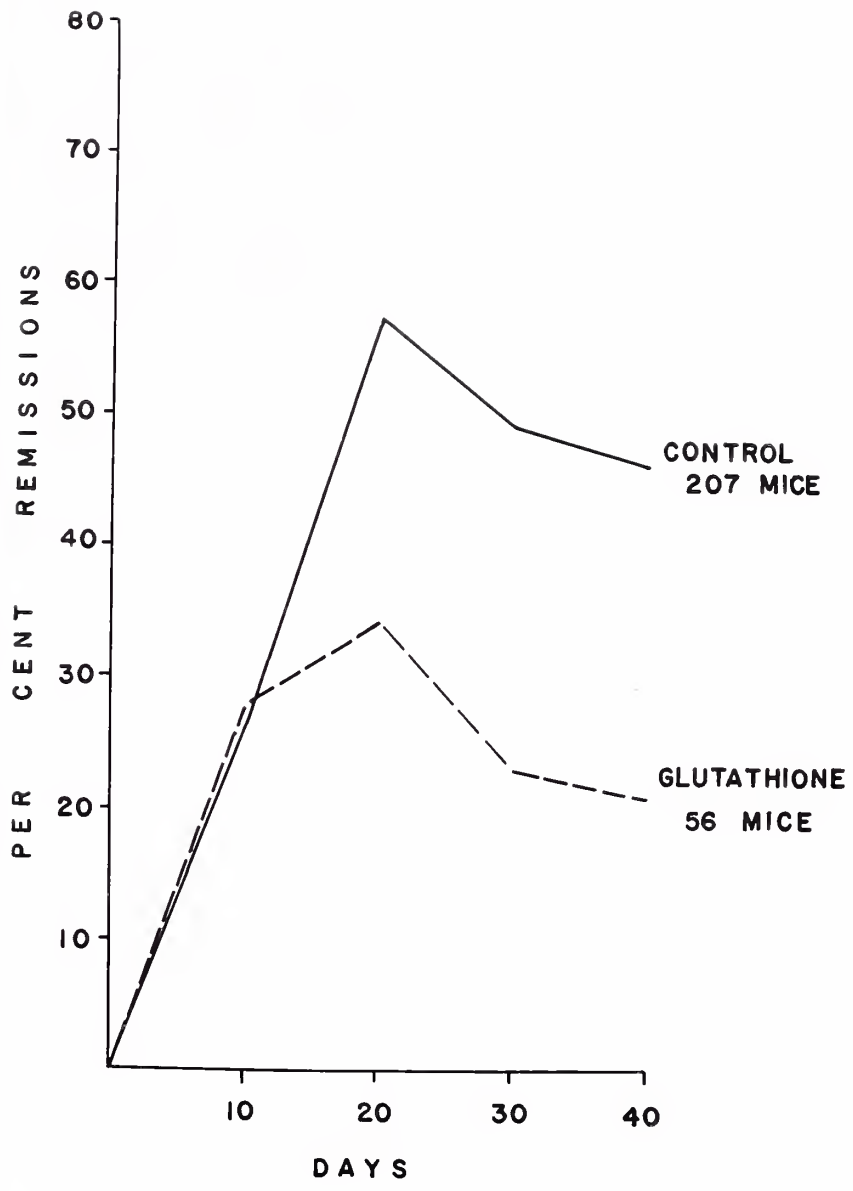
C. Chemical Agents Affecting Radiosensitivity

1. Glutathione

Previous investigators have demonstrated the protective effect of glutathione against radiation for normal tissues. However, this study was designed to study the effect of glutathione administration in a prototype clinical administration. This is essential in view of the fact that some sources have recommended administration of sulfhydrl compounds to patients undergoing radiation therapy to ward off "radiation sickness." A second purpose in the investigation was an attempt to create a radiation resistant tumor in order to search for substances which would reverse that resistance.

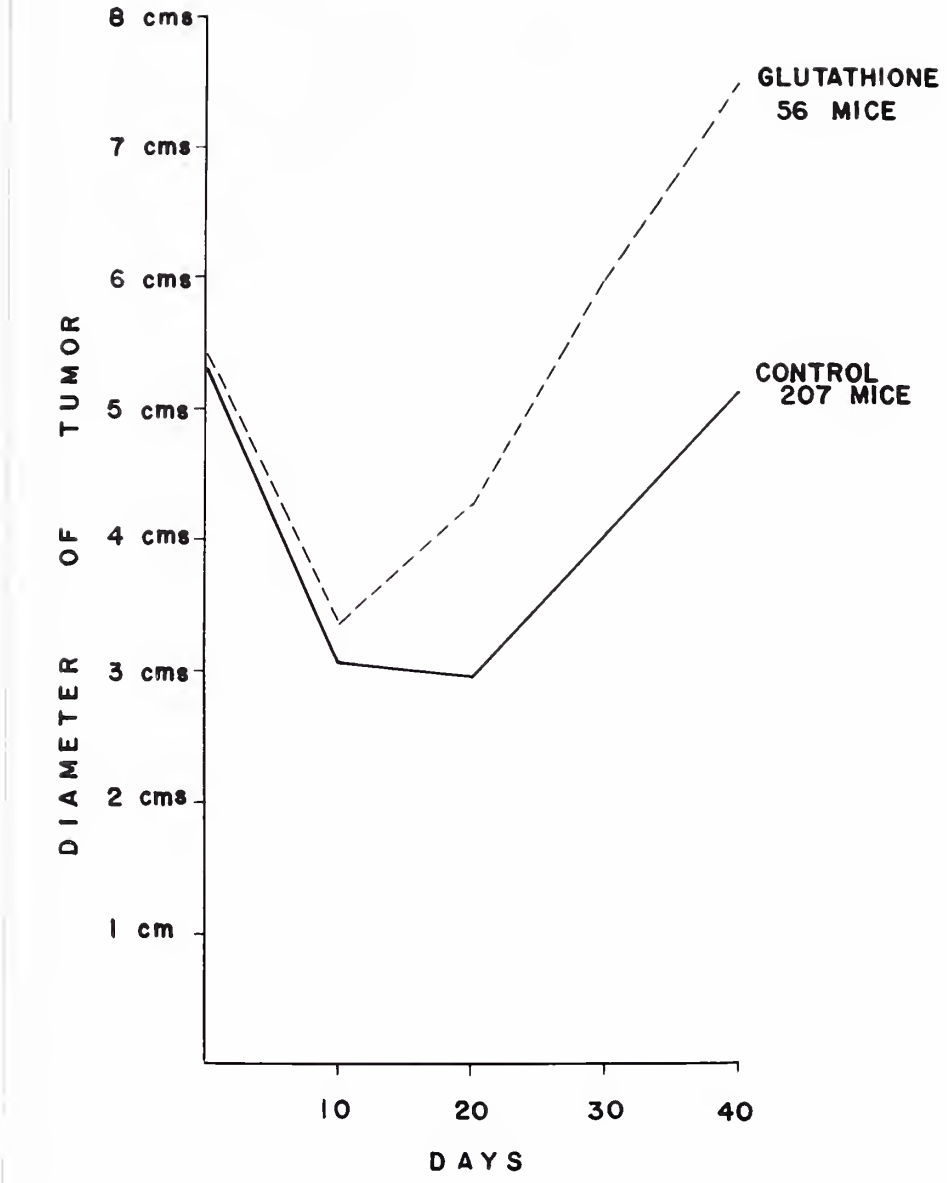
The results (see Graph VII) indicate a definite protection of the tumor from radiation therapy due to systemic glutathione administration. This protection of the tumor is statistically significant after ten days and is constant (chi square = 11 at forty days) throughout the experiment. The

Graph VII



Graph VII - Effect of glutathione and radiation versus radiation alone

Graph VIII



Graph VIII - Effect of glutathione and radiation versus radiation alone (on tumor size)

figures indicate administration of glutathione prior to radiation lowers the remission rate which can be expected of the tumor. They also indicate that this is a "radio-resistant" tumor with which further studies could be carried out.

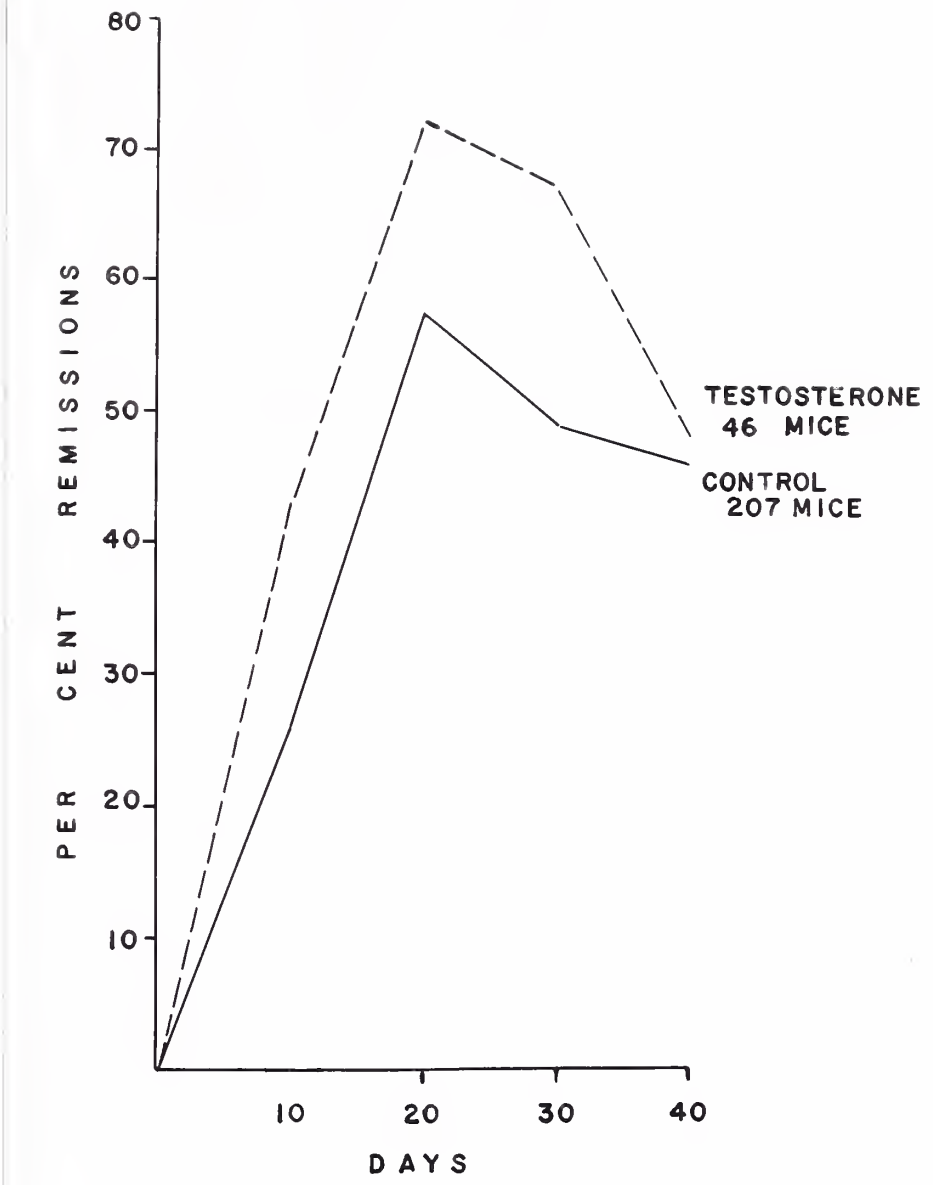
2. Testosterone

Graph IX gives the results of the administration of testosterone 24 hours prior to radiation on radiosensitivity of tumors. True to what would be expected from the Grahams' work, there is an early increased remission rate which is of borderline significance. However, in the later remission rate the curve falls almost to control levels. Thus if there is a radiation-enhancing effect of testosterone, under these conditions it is transient and difficult to show.

3. Testosterone and glutathione

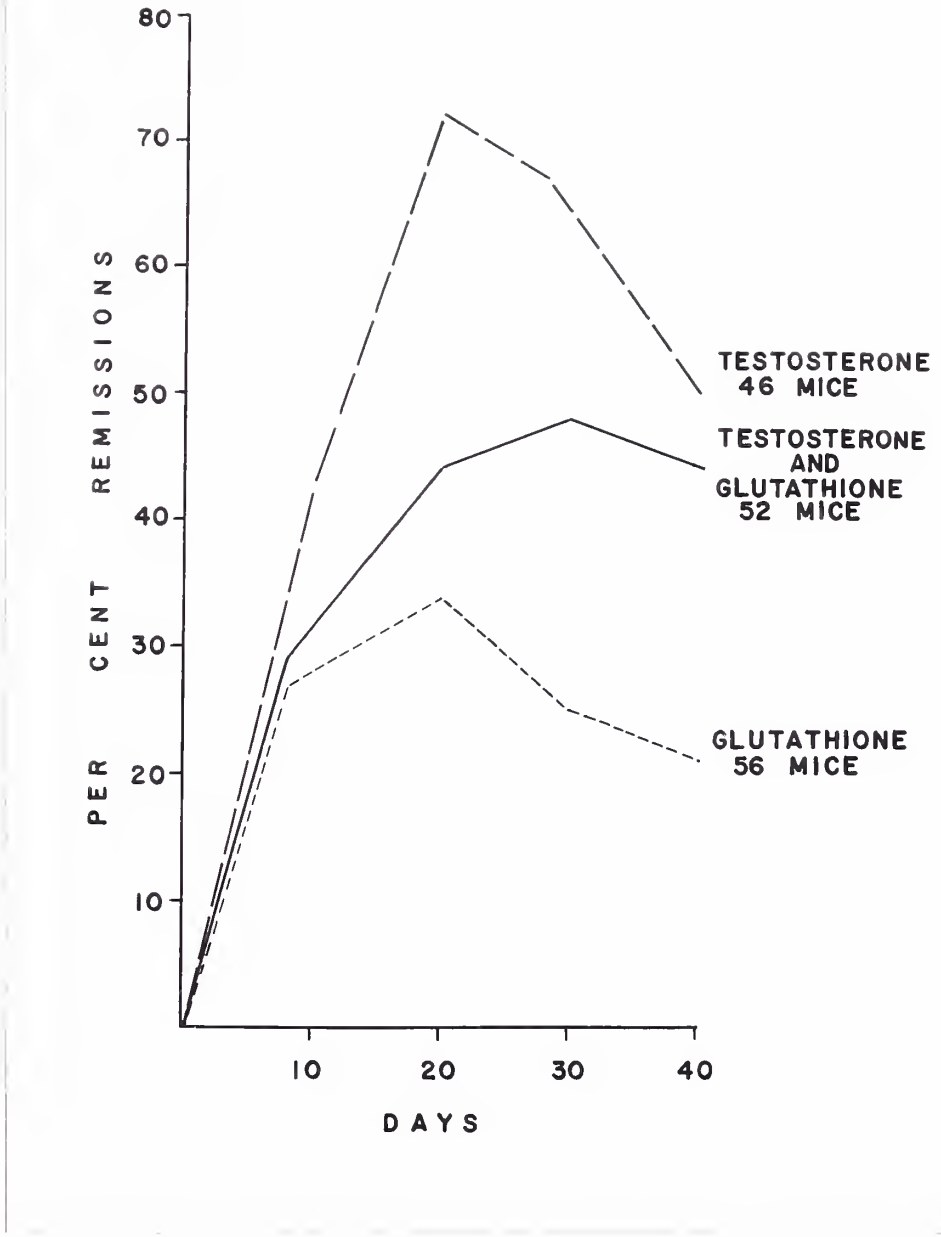
Combination of testosterone and glutathione were chosen since glutathione had been shown to make a tumor artifically radio-resistant. An attempt was made to reverse this resistance with testosterone. Graph X shows the results graphically. In the case of an artifically radio-resistant tumor, testosterone does reverse the radio-resistance bringing the remission rate up to that of controls. The difference is probably statistically

Graph IX



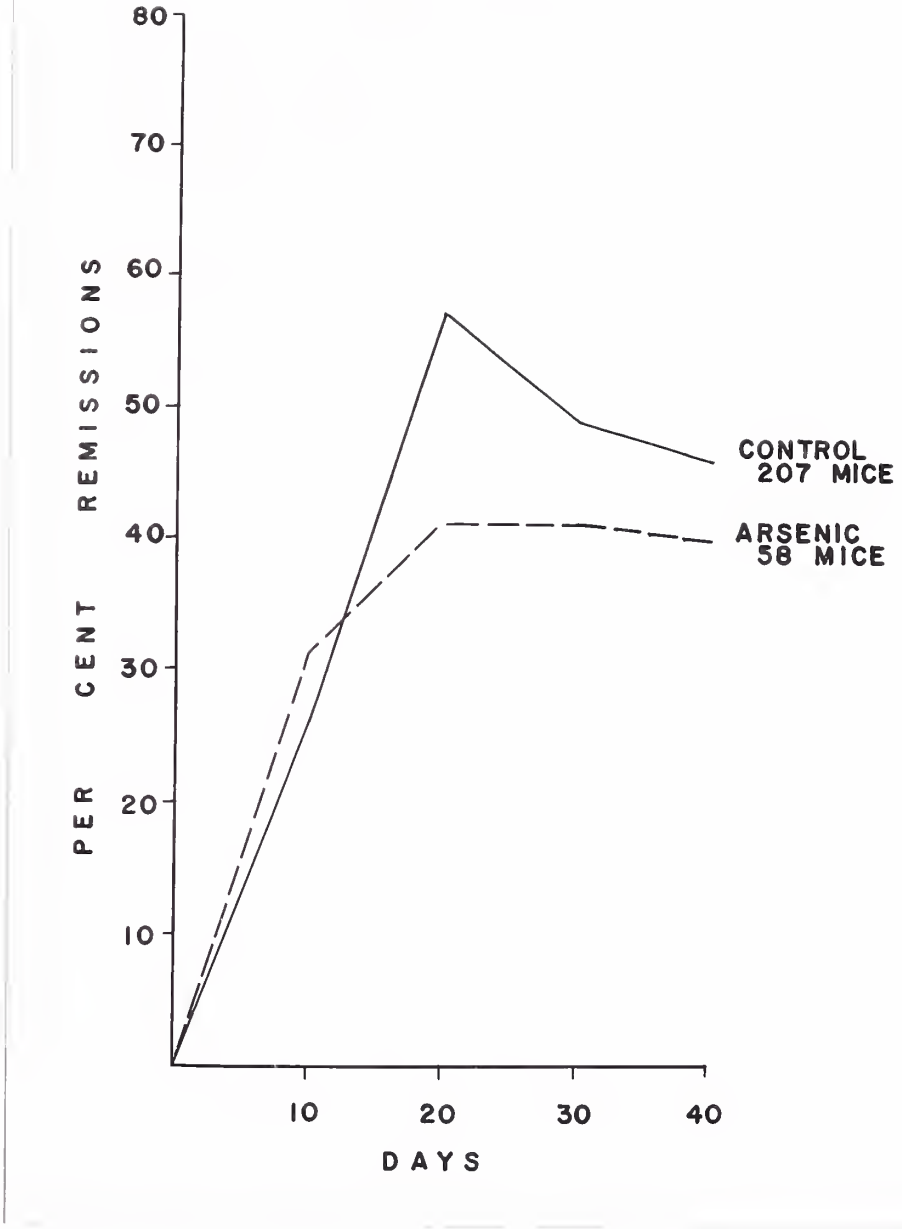
Graph IX - Effect of testosterone and radiation versus radiation alone

Graph X



Graph X - Effect of testosterone and glutathione versus testosterone alone and glutathione alone

Graph XI



Graph XI - Effect of arsenic and radiation versus radiation alone

valid with a chi square of 6. The protective effect of glutathione administration upon mice premedicated with testosterone is seen to closely parallel the protective effect on normal mice given glutathione.

These observations suggest testosterone administration prior to radiation might be useful in radio-resistant tumors if the mechanism of radio-resistance is an overabundance of SH compounds.

4. Fowler's solution

Potassium arsenite (Fowler's solution) was administered to tumor-bearing mice prior to radiation in hopes that by inactivating some SH substances, radio-sensitivity might be increased. The results were negative as shown in Graph XI. Arsenic-treated mice are shown to have a lower (borderline significant only at twenty days) remission rate than those of controls. The mortality in this group was somewhat heavier than in other experimental groups, although exact figures are not available.

Under the conditions enumerated, administration of potassium arsenite had no consistently significant effect on the radio-sensitivity of tumors.

Summary

1. Studies with transplanted mouse tumors provide a useful approach to the study of clinical radiation therapy problems.

2. Heat and testosterone temporarily improve remission rates in mouse tumors receiving radio-therapy.

3. Anoxia and anoxia plus cold protect tumor tissue against destruction by radio-therapy.

4. Incision one hour prior to radiation improves remission rates in mouse tumors receiving radiation therapy.

5. Glutathione prior to radiation lowers the remission rate in mouse tumors receiving radio-therapy. This effect can be negated by concommitant testosterone administration.

6. Fowler's solution and incision post radiation had no constant significant effect of remission rate of mouse tumors undergoing radiation therapy.

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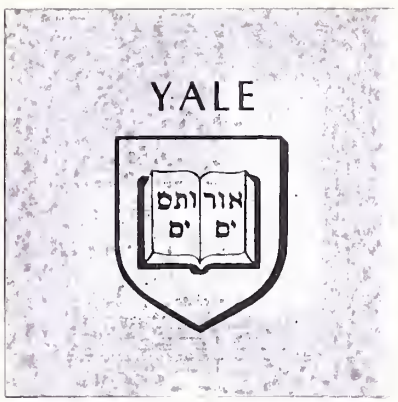
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